A linker region is an amino acid sequence that operably links two functional or structural domains of a protein, for example, between two metal binding domains of a chelon.

A nucleic acid construct is a nucleic acid molecule which is isolated from a naturally occurring gene or which has been modified to contain segments of nucleic acid which are combined and juxtaposed in a manner which would not otherwise exist in nature.

Nucleic acid molecule means a single- or double-stranded linear polynucleotide containing either deoxyribonucleotides or ribonucleotides that are linked by 3'-5'-phosphodiester bonds.

Two DNA sequences are operably linked if the nature of the linkage does not interfere with the ability of the sequences to effect their normal functions relative to each other. For instance, a promoter region is operably linked to a coding sequence if the promoter is capable of effecting transcription of that coding sequence in a particular host cell of choice.

A polypeptide is a linear polymer of amino acids linked by peptide bonds.

A promoter is a cis-acting DNA sequence, generally 80-120 base pairs long and located upstream of the initiation site of a gene, to which RNA polymerase may bind and initiate correct transcription. There can be associated additional transcription regulatory sequences which provide on/off regulation of transcription and/or which enhance (increase) expression of the downstream coding sequence.

A recombinant nucleic acid molecule, for instance a recombinant DNA molecule, is a novel nucleic acid sequence formed in vitro through the ligation of two or more nonhomologous DNA molecules (for example a recombinant plasmid containing one or more inserts of foreign DNA cloned into its cloning site or its polylinker).

Transformation means the directed modification of the genome of a cell by the external application of purified recombinant DNA from another cell of different genotype, leading to

its uptake and integration into the subject cell's genome. In bacteria, the recombinant DNA is not typically integrated into the bacterial chromosome, but instead replicates autonomously as a plasmid.

A vector is a nucleic acid molecule that is able to replicate autonomously in a host cell and can accept foreign DNA. A vector carries its own origin of replication, one or more unique recognition sites for restriction endonucleases which can be used for the insertion of foreign DNA, and usually selectable markers such as genes coding for antibiotic resistance, and often recognition sequences (e.g. promoter) for the expression of the inserted DNA. Common vectors include plasmid vectors and phage vectors.

The chelon specifically embodied by the present invention is an artificial protein comprising at least two metal binding domains derived from the MerR protein of the Tn21 *mer* (mercury resistance) operon. In the presence of mercuric ions, the MerR protein binds mercuric ions and serves as a positive regulator of the mercury resistance operon. An isolated metal binding domain polypeptide has been described [Zeng et al. (1998) *Biochemistry* **37**:15885-15895]. See also Caguiat et al. (1990 *J. Bacteriol.* **181**:3462-3471, for further discussion of heavy metal binding by the MerR protein and cadmium-binding variants.

The present work provides the first demonstration that an independent sub-domain of a naturally occurring protein can be incorporated in a heavy metal sequestration protein which binds cognate metal ions with high specificity and high affinity. Previously described metal binding proteins are those which bind divalent "beneficial" metal ions, e.g., nickel, copper and zinc. Generally for those proteins, the affinities and specificities for heavy metals are lower, although some forms of metallothionein bind divalent cadmium ions. The previously known metal binding proteins are subject to having the metal ligand competed off by naturally occurring thiols such as glutathione and cysteine. The relatively low affinities reflect the physiological roles of these proteins in serving as chaperones for the metal ions en route to the active sites of enzymes or structural sites of DNA binding proteins. The intact MerR protein and its metal binding domain have much higher affinity (about 9 orders of magnitude) than natural thiols such as glutathione or cysteine and nonphysiological thiols such as 2-

mercaptoethanol [Zeng et al. (1998) supra]. The wild-type MerR protein binds mercuric ion with an affinity of 10^{-9} M even in the presence of millimolar quantities of thiols, and it binds divalent cadmium and divalent zinc ions a hundred-fold and thousand-fold less well.

In the chelons of the present invention, the metal binding site requires that an antiparallel association of sequence motifs come together in a way that mimics the natural mercuric ion binding site of MerR dimers. The ability to bind metal ions by the chelon is documented in Figure 2.

Bacterial cells over-expressing a chelon (MBD) or MerR protein intracellularly bind more Hg than a strain carrying only the plasmid vector (Fig. 5). Western analysis indicates that the MerR and chelon proteins are produced in similar amounts. However, MerR binds Hg ions somewhat better than does the chelon protein. Without wishing to be bound by any particular theory, it is believed that this may arise because of differences in folding stability for the very short, artificial chelon compared to the full-length MerR protein inside the cell. Nonetheless, chelon protein expression clearly increases the amount of Hg bound.

When the chelon and MerR proteins are each attached to a solid substrate (Streptactin Sepharose) *in vitro* via StrepTag streptavidin/Streptactin sequences (Genosys) both bind the solid substrate with equal efficiency, i.e. with very similar affinity (in terms of motes of protein bound to the resin), but the bound chelon is more effective than bound MerR protein in binding Hg ions (Table 3). Although the Hg occupancy of the chelon is only 50%, its proficiency in this regard compared to MerR (which binds Hg with ca. 3-fold less efficiency) is expected because each tethered copy of the chelon contains a complete Hg binding site. However, for MerR, the tethered monomer must either capture a free monomer from solution or must contact a monomer bound to an adjacent site on the substrate surface in order to form the dimeric Hg binding site. Without wishing to be bound by theory, we believe that this "search" process limits the efficiency of tethered full length MerR as compared to the single polypeptide chelon in capturing Hg.